THE
BOTANICAL GAZETTE

INFLUENCE OF SALTS ON BACTERIAL ACTIVITIES OF SOIL

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Salts occurring naturally in soils and those applied to them in various operations influence the number, species, and activity of the soil microflora. These factors in turn are reflected by yields obtained. Some substances applied to a soil may serve as food for the growing plant; others increase plant growth indirectly. This latter effect may be due to the changing of the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified. This in turn may increase or decrease the available plant food of the soil. Other substances may react chemically with constituents within the soil, and in so doing liberate nutrients which may be utilized directly by the growing plant (24). Again, they may directly modify the microflora and microfauna of the soil both as to numbers and physiological efficiency. In some cases all three changes may be wrought by the same salt. In each case the soil is so changed that its crop-producing power is modified. The question arises, therefore, as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activity of the soil. Furthermore, there are millions of acres of land which contain varying amounts of soluble salts. Some of these soils contain such large quantities of the so-called "alkalis" that no vegetation grows upon them.

Other soils contain only a medium amount of soluble salts, and the vegetation consists chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts, and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition are problems confronting the soil chemist. These problems can be solved more successfully when the laws governing the influence of salts upon the growing plant and their action upon the chemical, physical, and biological properties of the soil are understood. This study was undertaken, therefore, with the hope of obtaining light on some of these laws. It was carried on with soils which naturally were productive, but

TABLE I

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO
RETARD AMMONIFICATION IN THE SOIL IN UNIT TIME

	Chloride	Sulphate	Nitrate	Carbonate
Calcium	156×10^{-7}	5×10^{-3}	78×10^{-7}	$11 \times 10^{-3*}$
Potassium	625×10^{-7}	125×10^{-6}	1×10^{-3}	6×10^{-3}
Iron	125×10^{-6}	625×10^{-7}	25×10^{-5}	6×10^{-3}
Manganese	125×10^{-6}	25×10^{-5}	5×10^{-4}	11×10^{-3}
Sodium	125×10^{-6}	5×10^{-4}	5×10^{-4}	6×10^{-3}
Magnesium	5×10^{-4}	5×10^{-4}	156×10^{-7}	1×10^{-3}

* Not toxic at 11×10^{-3}, highest concentration tested.

became unproductive through the addition of known quantities of various salts. This would give a soil which at first had the same physical, chemical, and biological properties, hence any difference found must be due to the salt added.

Using as a measure of toxicity of the various salts that quantity which if applied to a definite weight of soil reduces the ammonia produced in the soil as compared with a similar untreated soil kept under similar conditions, the values reported in table I were obtained. The quantity recovered from the soil by leaching was also determined, but for this study the quantity applied is used for the reason that the direct or indirect action of the addition of a specific salt is being determined and not the absolute point of toxicity, which undoubtedly will vary with different soils and conditions.

The soil used was a calcareous loam (8), rich in potassium, phosphorus, and all the essential elements except nitrogen, which was low, as was also the organic matter of the soil. The results as reported are the average of a great number of determinations, and represent rather accurately the toxic point of the various salts in this specific soil, hence the results represent the relative toxicity for several salts in this specific medium. This may or may not vary in a different medium. The results, therefore, are not to be taken as absolute but as relative, which can only justly be comparable with other results obtained under like conditions.

It is quite evident from these results that the toxicity of a compound is governed by both anion and cation. Without exception the chlorides are more toxic than the corresponding nitrates. The sulphate varies, depending upon the cation with which it is combined, whereas in every instance the carbonate is less toxic than any of the other salts. The relative toxicity for the anions, therefore, can be written in the order $Cl > NO_3 > SO_4 > CO_3$, thus indicating that the monovalent anions are more toxic than the divalent anions. It must be borne in mind, however, that there would be more anions in unit volume of the monovalent than of the divalent, where a divalent cation is combined with the monovalent anion, as these salts were tested on the basis of mole concentrations. When re-examined with equivalent ionic concentration, the difference, although small, still maintained the same order.

Excluding the difficultly soluble salts, and averaging the results for the cation, we obtain the series $Mn > Mg > Fe > Ca > Na > K$, or the divalent cation is found to be more toxic than the monovalent. Here also the number of ions would enter, and if the toxicity be due either to an osmotic or precipitant reaction the order is what would be expected.

Examining the results obtained with nitrification (13) on the same basis as has been done with ammonification, we obtain as the points of toxicity the results given in table II.

The most important conclusion to be drawn from these results as compared with the preceding is the greater sensitiveness of the nitrifying organisms to the different salts as compared with the

ammonifying organisms. There are three chlorides, one nitrate, and one sulphate which were slightly more toxic for nitrifiers than for ammonifiers. In most cases the resistance of the ammonifiers to salts is sufficiently greater that nitrification in a soil may be materially decreased without seriously interfering with ammonification. Moreover, the toxicity of salt increases with concentration much more rapidly toward nitrifiers than toward ammonifiers.

TABLE II

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD NITRIFICATION IN SOIL IN UNIT TIME

	Chloride	Nitrate	Sulphate	Carbonate
Calcium	78×10^{-7}	156×10^{-7}	$3 \times 10^{-3*}$	625×10^{-7}
Potassium	156×10^{-7}	312×10^{-7}	156×10^{-7}	156×10^{-7}
Iron	1×10^{-3}	312×10^{-7}	156×10^{-7}	625×10^{-7}
Manganese	312×10^{-7}	25×10^{-5}	1×10^{-3}	78×10^{-7}
Sodium	4×10^{-3}	156×10^{-7}	156×10^{-7}	156×10^{-7}
Magnesium	78×10^{-7}	78×10^{-7}	625×10^{-7}	78×10^{-7}

* Not toxic at 3×10^{-3}, highest concentration tested.

TABLE III

MOLES OF THE VARIOUS SALTS PER 100 GM. OF SOIL REQUIRED TO RETARD AZOFICATION IN SOIL

	Chloride	Sulphate	Nitrate	Carbonate
Sodium	$2 \times 10^{-3*}$	$2 \times 10^{-3*}$	$2 \times 10^{-3*}$	$2 \times 10^{-3*}$
Potassium	$2 \times 10^{-3*}$	1×10^{-3}	$2 \times 10^{-3*}$	125×10^{-6}
Calcium	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$
Magnesium	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$	1×10^{-3}
Manganese	$1 \times 10^{-3*}$	1×10^{-3}	$1 \times 10^{-3*}$	$1 \times 10^{-3*}$
Ferric	$5 \times 10^{-3*}$	$5 \times 10^{-3*}$	5×10^{-3}	5×10^{-3}

* Not toxic at this concentration, the highest tested.

The order of toxicity of the anion in the case of the nitrifiers is nearly the reverse of that for the ammonifiers. The order for nitrification is $CO_3 > NO_3 > SO_4 > Cl$, whereas the order of toxicity of the cation as measured in terms of nitrification becomes $K > Mg > Fe > Mn > Ca > Na$. It is evident, therefore, that both the anions and cations exert an influence in determining the toxicity of the salts.

Examining nitrogen fixation (12) in the same light the results given in table III are obtained.

Possibly potassium carbonate is more toxic to the nitrifiers of the soil than it is to the nitrogen-fixing organisms. It is certain that the azofiers in soil are much more resistant to these salts than are the other classes of microorganisms. The concentration of the salts in the soil was not high enough to permit conclusion as to the relative toxicity of the various anions and cations. The tests, however, make it certain that alkali soils which have a vigorous nitrifying or ammonifying microflora will maintain a vigorous azofying flora, even though they contain considerable quantities of soluble salts.

The influence of an anion upon the internal friction of colloids varies with concentration and reaction of medium, and it is interesting to note that the series for the ammonifiers is the same as that for acid solutions of proteins, whereas that for the nitrifiers is the order for these ions upon a neutral or alkaline solution of the protein. If, therefore, toxicity of these salts is due in a measure to their changing the internal viscosity of the protoplasm, we should have to assume a slight difference in the protoplasm making up the cell of the two groups of microorganisms, the one being electro-positive and the other electro-negative.

The order of toxicity of the cation to the ammonifiers is not far different from what would be expected if toxicity were due to a precipitation of the protoplasmic colloids. When the nitrifying series are examined in the light of the HOFMEISTER series, however, the potassium ion is found on the opposite extreme of the series from where it should be.

Whether this is due to analytical error or to the potassium ion being especially poisonous because of its changing the state of turgescence of the organic colloid is not clear. It would appear, however, that if the latter were the correct explanation, we might expect potassium to change positions in the ammonifying series. This would appear more reasonable, for both the ammonifiers and nitrifiers function normally in the same medium, and the same evolutionary forces have been at work bringing these organisms to their present condition.

The relative toxicity of the anions toward ammonifiers and the relative toxicity of the cation as measured in terms of both ammoni-

fication and nitrification point to the conclusion that toxicity is due in a large measure to osmotic influences. This conclusion is based on the assumption that the salts on passing into solution behave as they would in pure water, an assumption which is not warranted, for it is a well known fact that the addition of a salt to a soil causes an exchange of ions. This may either increase or decrease the total number of soluble particles in the soil solution, and hence correspondingly change the osmotic pressure of the soil solution. The logical procedure, therefore, is to determine the osmotic pressure (14) of the soil at the concentrations at which the various salts become toxic to the different classes of microorganisms. The results for such tests for the ammonifying series are given in table IV.

TABLE IV

Osmotic pressure of salt-treated soil toxic to ammonifying organisms

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium...............	1.62	2.77*	1.96	8.41*	1.79
Potassium............	1.53	1.89	1.75	9.05*	1.72
Calcium..............	1.43	1.53	2.45	1.61	1.75
Magnesium...........	1.62	1.68	1.88	1.80	1.75
Manganese...........	1.56	3.53*	1.78	2.04	1.79
Iron.................	2.34	2.71*	1.84	1.84	2.00
Average..........	1.68	1.70	1.94	1.84	....

* Not counted in the average.

Sixteen out of the twenty-four salts become toxic when the osmotic pressure ranges between 1.43 and 1.96 atmospheres. The average for the cation shows a variation of from 1.72 to 2.00 atmospheres, and in all except the iron ranges between 1.72 and 1.79, a difference of only 0.05 of an atmosphere. The anion shows a variation from 1.68 to 1.94. The small variation in osmotic pressure among the various salts at which they become toxic indicates that the osmotic pressure is an important factor in determining toxicity.

We find that when the ammonia produced had been reduced by about 10 per cent, the average osmotic pressure of the soil solution was 2.55 atmospheres; when the ammonia was reduced 25 per cent, the osmotic pressure was 5.49 atmospheres; and when ammonia

produced was 50 per cent of normal, the osmotic pressure was 9.5 atmospheres. Table VI gives these data for both ammonification and nitrification.

The nitrifiers, therefore, are more sensitive to osmotic changes than are the ammonifying organisms, but in both cases the toxicity increases more rapidly than does the osmotic pressure. This is very rapid in the case of the nitrifiers.

TABLE V

OSMOTIC PRESSURE OF SALT-TREATED SOIL TOXIC TO NITRIFYING ORGANISMS

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium...............	4.25*	1.81	1.80	1.84	1.82
Potassium............	1.53	1.67	1.75	1.49	1.61
Calcium..............	1.60	1.54	....	1.54	1.56
Magnesium...........	1.81	1.59	1.88	1.79	1.78
Manganese...........	1.65	2.73*	2.22	2.01	1.96
Iron.................	4.94*	1.89	1.85	1.86	1.87
Average...........	1.65	1.70	1.90	1.85	....

* Not counted in the average.

TABLE VI

REDUCTION OF PRODUCT	OSMOTIC PRESSURE TO REDUCE	
	Ammonification 1 per cent	Nitrification 1 per cent
10 per cent................	0.255 atmospheres	0.197 atmospheres
25 per cent................	0.199 atmospheres	0.117 atmospheres
50 per cent................	0.190 atmospheres	0.088 atmospheres

The average for the anions in the case of the nitrifiers is within experimental error, the same as that for the ammonifiers. The average for the cation is slightly lower for the nitrifiers than for the ammonifiers, but quite similar in both series, thus indicating that the toxicity in both sets is governed by the same factor, possible osmotic pressure plus a physiological effect produced by the salt. The physiological influence may be due to the replacing of ion in the living protoplasm, thus changing its physical, chemical, and electrical properties so that they are incompatible with life. It is thus assumed with LOEB (18) that the toxicity of sodium salts on entering the cell is due to the formation of sodium proteinates,

which, if present in too great a proportion in the cell, confer upon the protoplasm properties which are incompatible with the maintenance of normal functioning. The toxicity of calcium salts is likewise attributed to an undue predominance of calcium proteinate in the cell. An admixture of several types of protein salts is required to give the protoplasm of the cell the exact qualities essential to the maximal furtherance of its vital activities. Two factors, therefore, may enter in the toxicity of a salt: (1) the permeability of the salt for the living protoplasm, and (2) the chemical, physical, or electrical influence of the salt upon the protoplasm after entering the cell.

Those cases in which the osmotic influence is the predominating factor should show a marked decrease in toxicity when the solution is diluted (11); whereas in those cases in which the physiological effect predominates, the addition of another salt which would increase osmotic pressure may show a decrease in toxicity, due to a physiological balancing of the solution.

Numerous experiments have shown that the relative toxicity of sodium chloride, sodium carbonate, potassium carbonate, and calcium carbonate, as measured in terms of ammonification, decreases as the water added to a soil increases. All the other salts become relatively more toxic, thus indicating that some factor in addition to osmotic pressure is entering. In this regard the nitrifying organisms act quite differently, and toxicity is neutralized with potassium chloride, potassium sulphate, magnesium nitrate, and magnesium chloride, but not with the remaining salts when water is added. Were osmotic pressure alone the disturbing factor, it would be impossible to neutralize the toxic action of one salt by the addition of another, thus increasing osmotic pressure and at the same time decreasing toxicity.

This conception of antagonism and balanced solutions was first applied to a study of bacteria by LIPMAN in 1909. In his experiments (20) on the rate of ammonification of *Bacillus subtilis*, he showed that there is some antagonism between sodium and magnesium. On the other hand, he (21) found no antagonism, but increasing toxicity, when magnesium and calcium were com-

bined. Later he (22) demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium carbonate, and between sodium sulphate and sodium carbonate, thus indicating that the anions as well as the cations at times may play a part in antagonism.

KELLEY (16), in studying the ammonification and nitrification of certain soils, found no antagonism between magnesium and sodium. LIPMAN and BURGESS (23), however, observed in the case of nitrogen fixation by *Azotobacter chroococcum* an antagonism between sodium and magnesium.

WINSLOW and FALK (39) have observed antagonistic effects in experiments on *Bacillus coli*. They found that cultures suspended in solutions of sodium chloride or calcium chloride were decreased in number, that higher concentrations produced sterilization of the culture, and that a combination of sodium chloride and calcium chloride in the molecular proportions of 5 to 1 was favorable to the growth of the organisms.

SHEARER (29, 30) also demonstrated similar effects of salts upon the viability of *Meningococcus* and *Bacillus coli*. He found that a combination of sodium chloride and calcium chloride was favorable to growth, whereas each salt used separately retarded growth.

BROOKS (1) found that, as measured by the rate of respiration of *Bacillus subtilis*, there is a marked antagonism between sodium chloride and calcium chloride, and between potassium chloride and calcium chloride. The antagonism between sodium chloride and potassium chloride is slight, and the antagonism curve shows two maxima. Later, using the same method and organism, he (2) found a well marked antagonism between magnesium chloride and sodium chloride, and, contrary to the findings of LIPMAN (21), a very slight antagonism between magnesium and calcium. This is in keeping with my own (9) experience, which showed that a true antagonism exists between calcium sulphate *vs.* sodium carbonate, calcium sulphate *vs.* sodium nitrate, calcium sulphate *vs.* sodium sulphate, calcium sulphate *vs.* calcium chloride, calcium sulphate *vs.* magnesium sulphate, as measured in terms of ammonification.

This clearly indicates that the anion as well as the cation plays a part in antagonism. A similar antagonism exists between these salts as measured in terms of nitrification.

Furthermore, using the ammonia produced as the criterion, an antagonism is seen to exist between sodium sulphate *vs.* iron sulphate, calcium chloride *vs.* iron sulphate, sodium chloride *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron nitrate, sodium chloride *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, sodium nitrate *vs.* iron chloride, calcium chloride *vs.* iron chloride, sodium carbonate *vs.* iron nitrate, sodium carbonate *vs.* iron carbonate, sodium sulphate *vs.* iron nitrate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron nitrate, sodium nitrate *vs.* iron sulphate, magnesium sulphate *vs.* iron chloride, and magnesium sulphate *vs.* iron carbonate. This was small in the case of the first pair, and increased in the order named until the last, which neutralized 75 per cent of the toxic effect of magnesium sulphate.

As measured in terms of nitrification, a true antagonism was found to exist between sodium carbonate *vs.* iron carbonate, sodium chloride *vs.* iron chloride, magnesium sulphate *vs.* iron nitrate, sodium carbonate *vs.* iron sulphate, sodium nitrate *vs.* iron sulphate, sodium sulphate *vs.* iron carbonate, calcium chloride *vs.* iron carbonate, sodium nitrate *vs.* iron carbonate, sodium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron carbonate, sodium nitrate *vs.* iron chloride, sodium sulphate *vs.* iron nitrate, sodium sulphate *vs.* iron chloride, magnesium chloride *vs.* iron carbonate, calcium chloride *vs.* iron nitrate, magnesium sulphate *vs.* iron chloride, sodium chloride *vs.* iron sulphate, magnesium chloride *vs.* iron chloride, sodium carbonate *vs.* iron chloride, and magnesium chloride *vs.* iron nitrate. This was low in the case of the first pair, and increased progressively in the order named up to the last named pair, in which the iron nitrate increased the nitrification 420.7 per cent over that soil treated with magnesium chloride alone.

The results, therefore, indicate the toxicity of soluble salts toward soil microorganisms to be due to an osmotic effect which makes it impossible for the cell to take up its normal nutrients,

but permits foreign or unbalanced constituents to enter. These foreign or unbalanced salts on entering the cell protoplasm interact with the proteins thereof, forming within the living protoplasm foreign proteinates, the physical, chemical, and electrical properties of which are different from those of the normal protoplasm. Hence we have the protoplasm rendered incapable of normal functioning. The first effect is governed to a marked extent by the osmotic pressure of the medium in which the organism is functioning, and the second by the specific salt, acid, or base which comes in contact with the protoplasm.

STIMULATING ACTION OF SALTS.—Many salts when added to a medium in which bacteria are functioning first stimulate, and as the concentration is increased the specific salt becomes toxic. The

TABLE VII

PERCENTAGES OF AMMONIA PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium...............	106.0	107.8	100.0	110.1
Potassium............	100.0	102.2	100.0	108.9
Calcium.............	100.0	100.0	103.2	114.6
Magnesium...........	100.7	100.0	104.5	103.5
Manganese...........	100.6	116.0	123.8	111.2
Iron................	118.6	102.9	103.9	107.9

extent of this stimulation varies with the salt, the concentration of the salt, the medium in which it is used, and the specific micro-organism grown upon it. If the ammonia produced in unit time in untreated soil is taken as 100 per cent, we obtain the values given in table VII for the various salts.

All except six of the salts stimulate bacterial activity. There is a wide variation, however, depending upon the specific salt. The cations arranged in a descending order would be $Mn > Fe > Na > Ca > K > Mg$. Although there is a wide variation, depending upon the cation and anion, it is interesting to note that it is not these elements which are to be recognized as plant foods, but the catalizers which head the list. This also appears in the case of the anion where the series is $SO_4 > Cl > CO_3 > NO_3$. The cations would appear to play a greater part as bacterial stimulants than do the

anions. This indicates a very marked acceleration of the speed with which the protein is transferred into ammonia, which would result in a greater available supply of this compound for the action of the nitrifiers. The speed with which the ammonia is oxidized to nitric acid also increases with the addition of various salts, as is brought out in table VIII.

TABLE VIII

PERCENTAGES OF NITRIC NITROGEN PRODUCED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium...................	142.0	101.0	100.0	100.0
Potassium................	106.5	106.4	100.0	100.0
Calcium..................	100.0	102.1	196.7	100.0
Magnesium...............	123.2	106.5	101.2	140.7
Manganese...............	112.9	125.4	113.2	108.4
Iron.....................	128.4	100.0	102.0	117.4

TABLE IX

PERCENTAGES OF NITROGEN FIXED IN SOIL TO WHICH VARIOUS SALTS WERE ADDED, THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate	Average
Sodium................	102.6	102.7	104.4	107.3	104.2
Potassium.............	100.0	101.3	105.0	100.0	101.6
Calcium...............	167.6	109.9	102.6	103.3	104.0
Magnesium.............	102.2	101.9	101.7	101.9	101.9
Manganese.............	102.3	102.2	102.4	100.0	101.7
Iron..................	104.3	102.0	100.4	101.8	102.1
Average............	101.9	103.3	102.7	102.3	....

All but six of these salts increase the accumulation of nitrates in the soil. Two which did not stimulate the ammonifiers also failed to stimulate the nitrifiers. In four cases there was no agreement between ammonifiers and nitrifiers. The manganese and iron salts are both strong stimulants, as was the case with the nitrifiers; potassium, which is an essential element, is the least effective of the cations, whereas chlorine is one of the most active, being exceeded in activity only by the sulphate ion.

It is evident that the nitrogen available to higher plants would be very materially increased through the addition of salts to a soil,

and furthermore, it is usually those salts which are considered soil amendments that exert the greatest influence. What effect would these salts have on the total nitrogen of the soil? This is answered in table IX.

Again potassium is least efficient, while sodium is one of the most efficient. It is interesting to note that the nitrates, which are usually stated as retarding azofication, stimulate it to a greater degree than any of the other compounds. In this respect calcium nitrate is more efficient than any other salt. This probably indicates that it is best to add nitrates to the soil in this form. It could not be claimed that this compound was carrying available calcium to the soil, for this soil already contains some 40 per cent of calcium and magnesium carbonate.

It is evident that these salts would increase both total and available nitrogen in the soil. They would also increase the crop-producing power in another way, namely, by increasing both directly and indirectly the available potassium and phosphorous of the soil (10).

SOLVENT ACTION OF BACTERIA.—BROWN (3) found that twelve out of twenty-three bacteria isolated from soil exerted a definite solvent action on difficultly soluble plant food. One organism which produced no gas, but a large amount of acid, exerted the greatest solvent action upon calcium carbonate; while other organisms which produced gas (largely carbon dioxide), but not as much acid as the former, gave an action more marked than of the stronger acid producer upon the dicalcium and tricalcium phosphates. *Bacillus subtilis*, *B. mycoides*, *B. proteus vulgaris*, and *B. coli communis*, as well as several agar cultures from garden soil, were found (26) to be capable of dissolving the phosphates of bone and to a less extent those of mineral phosphates. The greatest solvent action was exerted in media containing sodium chloride, potassium sulphate, and ferrous sulphate. Even yeast (17) may play an important part in dissolving phosphates. KROBER, however, considers that the life activity of the bacteria, that is, assimilation of phosphorus by the living organism, plays little or no direct part in solution of the phosphates, but that the latter is due to the action of the organic acid and of the carbon dioxide produced.

The acids produced by bacteria act upon various phosphates, changing them to the soluble monophosphate, but the rate of solution varies widely with the different compounds. Tricalcium phosphate, in precipitated form, dicalcium phosphate, and tetracalcium phosphate of Thomas slag are much more rapidly dissolved than the crystalline or the so-called amorphous phosphates. The general reaction is as follows: $2RCOOH + Ca_3(PO_4)_2 = Ca_2H_2(PO_4)_2 + (RCOO)_2Ca$. The reaction takes place most rapidly in soils containing large quantities of organic matter due to the active fermentation taking place in such soils.

GRAZIA (6) considers enzyme action to play a part in the dissolving of phosphates in soil, for he found the addition of chloroform to a soil reduced bacterial activity and decreased the acid produced, but at the same time the solution of phosphates was increased. This is in keeping with the finding of BYCHIKHIN and SKALSKI (4).

The presence of ammonium chloride and sulphate in the cultural media is especially effective, according to PEROTTI (25), in increasing the solvent action of bacteria for phosphorus. PEROTTI considers the successive steps in the solution or decomposition of phosphorus in bacteria cultures to be as follows: (1) generation of acids, (2) secondary reactions in the solution, and (3) production of a soluble phosphorus containing organic substance. The first two of these are the result of the activity of the bacteria on the phosphorus, and the last is due to the metabolic assimilation of the microorganisms.

The oxidation of sulphur by soil bacteria at times may generate sufficient acid to play a very important rôle in the dissolving of soil phosphorus. HOPKINS and WHITING (15), however, consider that the nitrosomonus are of first importance in rendering phosphorus and calcium soluble, due to the nitrous acid produced from ammonia: $(NH_4)_2CO_3 + 6O = 2HNO_2 + H_2CO_3 + 2H_2O$. The resulting nitrous acid reacts with the raw rock phosphate rendering it soluble, thus: $Ca_3(PO_4)_2 + 4HNO_2 = CaH_4(PO_4)_2 + 2Ca(NO_2)_2$.

Analyses showed that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized, aside from the action of the acid radicles asso-

ciated with the ammonia. The carbonic acid would play an important part also in this reaction: $4H_2CO_3 + Ca_3(PO_4)_2 = 2 Ca(HCO_3)_2 + CaH_4(PO_4)_2$. They found that neither ammonifying bacteria nor nitrobacter liberated appreciable quantities of soluble phosphorus from insoluble phosphates. Whereas this would readily occur in soil poor in calcium carbonate, yet in those rich in calcium carbonate there would be only small quantities of phosphorus liberated. This is the conclusion reached by KELLEY, but where the soluble phosphorus is rapidly being removed by the growing plant, there is little doubt but that the various soil organisms play an important part in rendering phosphorus soluble.

Moreover, it is quite evident that *Azotobacter* in their metabolism transform soluble inorganic soil constituents either into soluble or insoluble organic forms. This is especially true of phosphorus which is found in the ash of these organisms in such large quantities. The phosphorus, on the death of the organism, is returned to the soil in a readily available form, for STOKLASA has found that 50 per cent of the nitrogen of these organisms is nitrified within six weeks, and there is no reason for believing that the phosphorus would be liberated more slowly. There is also the possibility that many of the constituents of the bacterial cell may become available through the action of autolytic enzymes without the intervention of other bacteria (19).

It is further evident that an organism which possesses the power when growing under appropriate conditions of generating 1.3 times its own body weight of carbon dioxide during twenty-four hours (34) must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent, and will attack even ordinary quartz rock. Granite and rocks related to it are rather quickly attacked with the liberation of potassium and other elements. Likewise, it would act upon the tricalcium phosphate of the soil with the formation of more readily soluble phosphates, for this substance is four times as soluble in water charged with carbon dioxide as it is in pure water: $Ca_3(PO_4)_2 + 2CO_2 + 2H_2O = Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$. Moreover, the nitrogen-fixing organisms produce, among other products, formic, acetic, lactic, butyric, and other acids. The

kind and quantity of each depend upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble plant food which may be rendered soluble, for they have a high solvent power for the insoluble phosphates (32). The resulting salts of calcium would be attacked further by bacteria with the formation of calcium carbonate (5).

Whether these processes will give rise to an increase in the water-soluble plant food of the soil will depend upon whether the products of the second, the analytical reactions, exceed the products of the first, the synthetic reactions. We must not lose sight of the fact that although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they at first were derived through bacterial activity. This being the case, variations may be expected in the results reported from laboratory tests. STOKLASA (33) found that bacterial activity rendered the phosphorus of the soil more soluble, whereas SEVERIN (27) in his early work found the opposite to be true. Others have found that the solvent action of bacteria for insoluble phosphates is in direct proportion to the acid secreted by the organism (26).

In a later work SEVERIN (28) obtained different results. He used three soils, one sterile, a second sterilized and inoculated with pure cultures of *Azotobacter*, and a third sterilized and inoculated with cultures of *B. radicicola* and *Azotobacter*. The solubility of the phosphorus increased 8 to 14 per cent over that in the sterile soil. The acid-producing organisms, due to the acid secreted and their intimate contact with the soil particles, possess the power of dissolving silicates. Moreover, arsenic greatly stimulates nitrogen fixation, and there is a relationship between this increased bacterial activity and the form and quantity of phosphorus found in such a soil (7).

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers (35). There are cases, however, in which bacterial activity has decreased the water-soluble phosphorus of

the soil and of raw rock phosphate (36, 37). This, however, does not indicate that it is less available, for, as pointed out by TRUOG (38), the mixing of floats with manure caused an immediate decrease in the solubility of the phosphorus in 0.2 per cent citric acid solution, yet when thoroughly mixed with the feeding area of the soil its availability was increased to such an extent that some species of plants apparently were able to secure almost an adequate supply of phosphorus from this material. The addition of manure to the soil greatly increased the carbon dioxide production, and for a short time measurably increased the solvent action on floats. Where there is a decrease for a time of water-soluble phosphorus in fer-

TABLE X

TOTAL WATER-SOLUBLE PHOSPHORUS PLUS ORGANIC PHOSPHORUS IN SOIL TO WHICH
VARIOUS SALTS HAD BEEN APPLIED AND LEFT FOR THREE WEEKS,
THE UNTREATED SOIL BEING TAKEN AS 100 PER CENT

	Chloride	Nitrate	Sulphate	Carbonate
Sodium...............	124.0	99.8	....	105.3
Potassium..............	106.6	105.7	....	102.1
Calcium...............	101.6	105.9	88.4	99.1
Magnesium.............	103.1	105.3	108.4	85.2
Manganese.............	99.3	86.1	101.1	135.1
Iron...................	100.2	103.4	118.3	92.5

menting media, it is probably due to the formation of phosphoproteids within the bodies of the bacteria (31), and these would later be rendered soluble, due either to further bacterial activity or autolytic enzymes. This increased bacterial activity should and actually does result in an increased water-soluble and organic phosphorus of the soil, as may be seen from table X, in which the water-soluble phosphorus plus the organic phosphorus in the untreated soil has been taken as 100 per cent.

In all except seven cases where the salts had increased bacterial activities there also resulted an increase in the available phosphorus. Moreover, associated with this increase of available phosphorus go increased crops as found in field and pot experiments, and the significant feature of these facts is that there is also an increase in the phosphorus of these plants (7, 10).

Summary

Many salts when applied to a soil in small quantities increase the bacterial activities of that soil. This is manifest by an increased production of ammonia, nitrates, and soluble and organic phosphorus, together with an increased nitrogen fixation. Usually, although not always, those salts which become toxic in the lowest concentration are the greatest bacterial stimulants.

There is a very close correlation between toxicity of the various salts and the osmotic pressure produced in the soil, thus showing that toxicity is due in part to osmotic disturbances. Another factor of equal importance is the change in chemical composition of the protoplasm resulting from the formation of salts of the protein other than those normally occurring in the living protoplasm, thus incapacitating them for their normal functions.

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